

New Staphylokinase Derivatives
Desiré José Collen
Atty. Docket No. 702-001525

1														14
Ser	Ser	Ser	Phe	Asp	Lys	Gly	Lys	Tyr	Lys	Lys	Gly	Asp	Asp	
15														28
Ala	Ser	Tyr	Phe	Glu	Pro	Thr	Gly	Pro	Tyr	Leu	Met	Val	Asn	
29														42
Val	Thr	Gly	Val	Asp	Ser	Lys	Gly	Asn	Glu	Leu	Leu	Ser	Pro	
43														56
His	Tyr	Val	Glu	Phe	Pro	Ile	Lys	Pro	Gly	Thr	Thr	Leu	Thr	
57														70
Lys	Glu	Lys	Ile	Glu	Tyr	Tyr	Val	Glu	Trp	Ala	Leu	Asp	Ala	
71														84
Thr	Ala	Tyr	Lys	Glu	Phe	Arg	Val	Val	Glu	Leu	Asp	Pro	Ser	
85														98
Ala	Lys	Ile	Glu	Val	Thr	Tyr	Tyr	Asp	Lys	Asn	Lys	Lys	Lys	
99														112
Glu	Glu	Thr	Lys	Ser	Phe	Pro	Ile	Thr	Glu	Lys	Gly	Phe	Val	
113														126
Val	Pro	Asp	Leu	Ser	Glu	His	Ile	Lys	Asn	Pro	Gly	Phe	Asn	
127														136
Leu	Ile	Thr	Lys	Val	Val	Ile	Glu	Lys	Lys					

Figure 1

09728670-032604

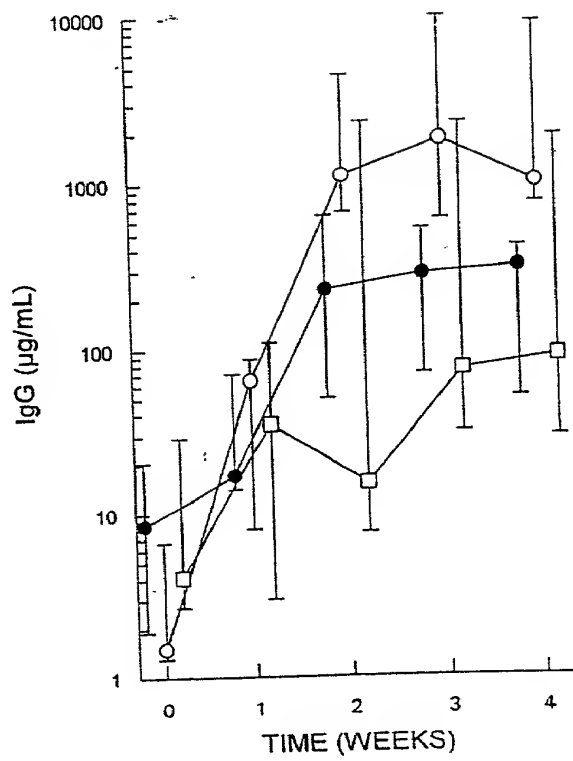
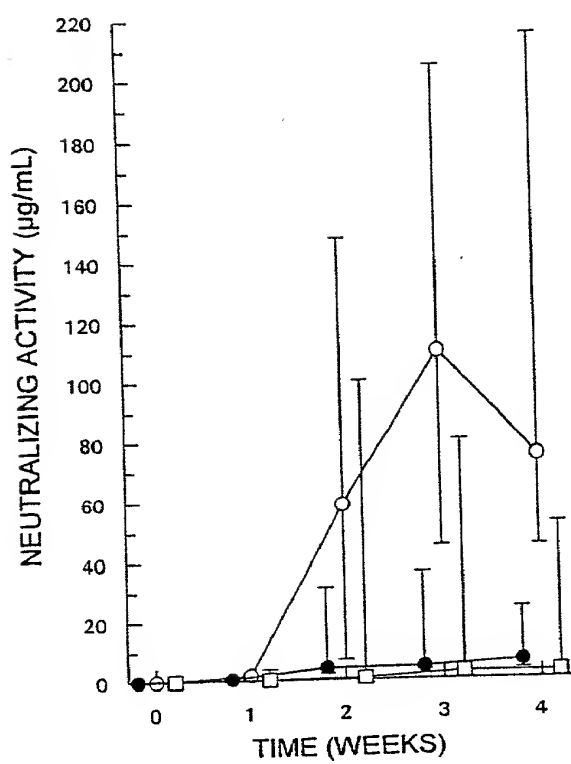
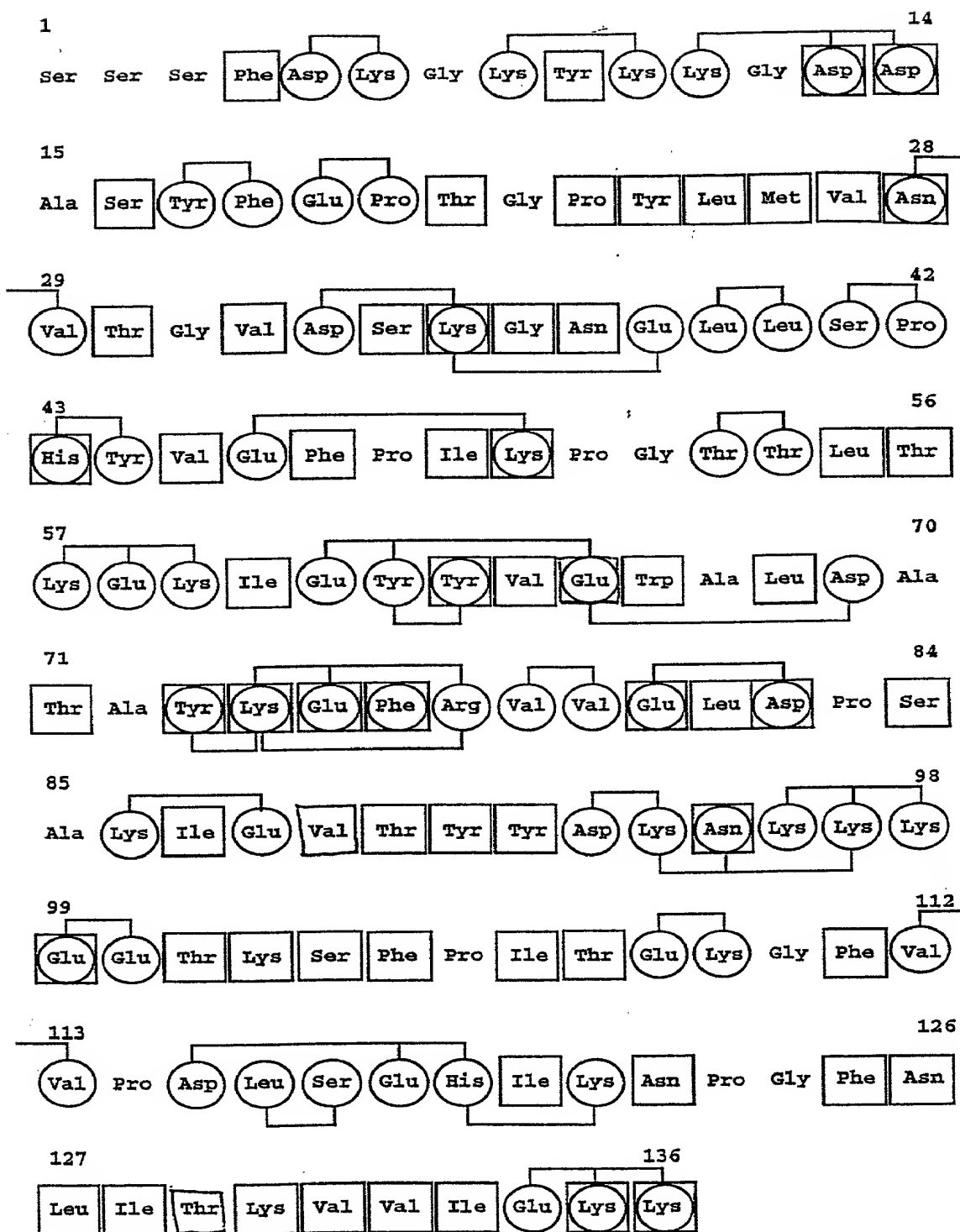


Figure 2



□ : single amino acid to Ala substitution
 ○ : combined (2 or 3) amino acid to Ala substitutions

Figure 3

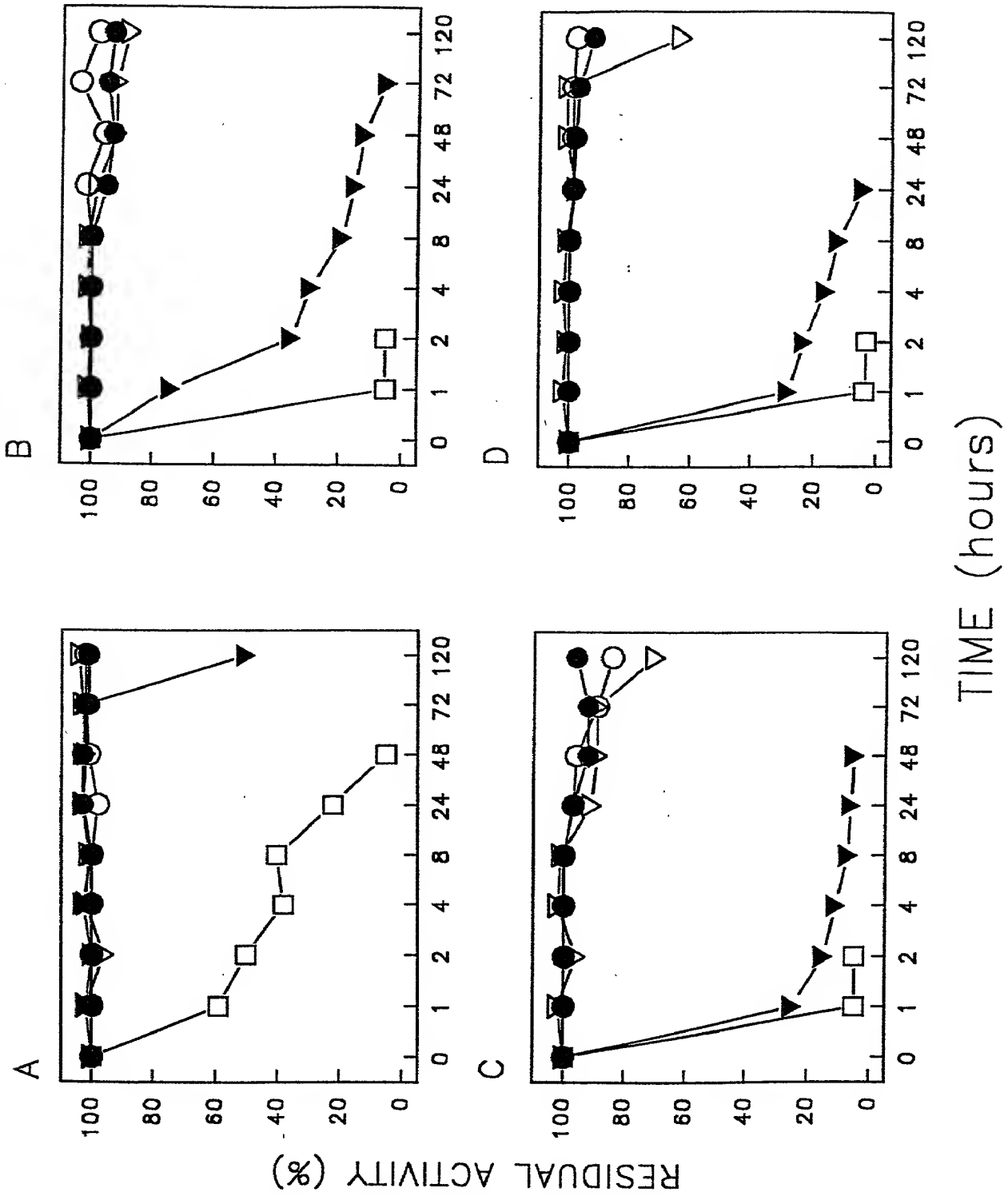
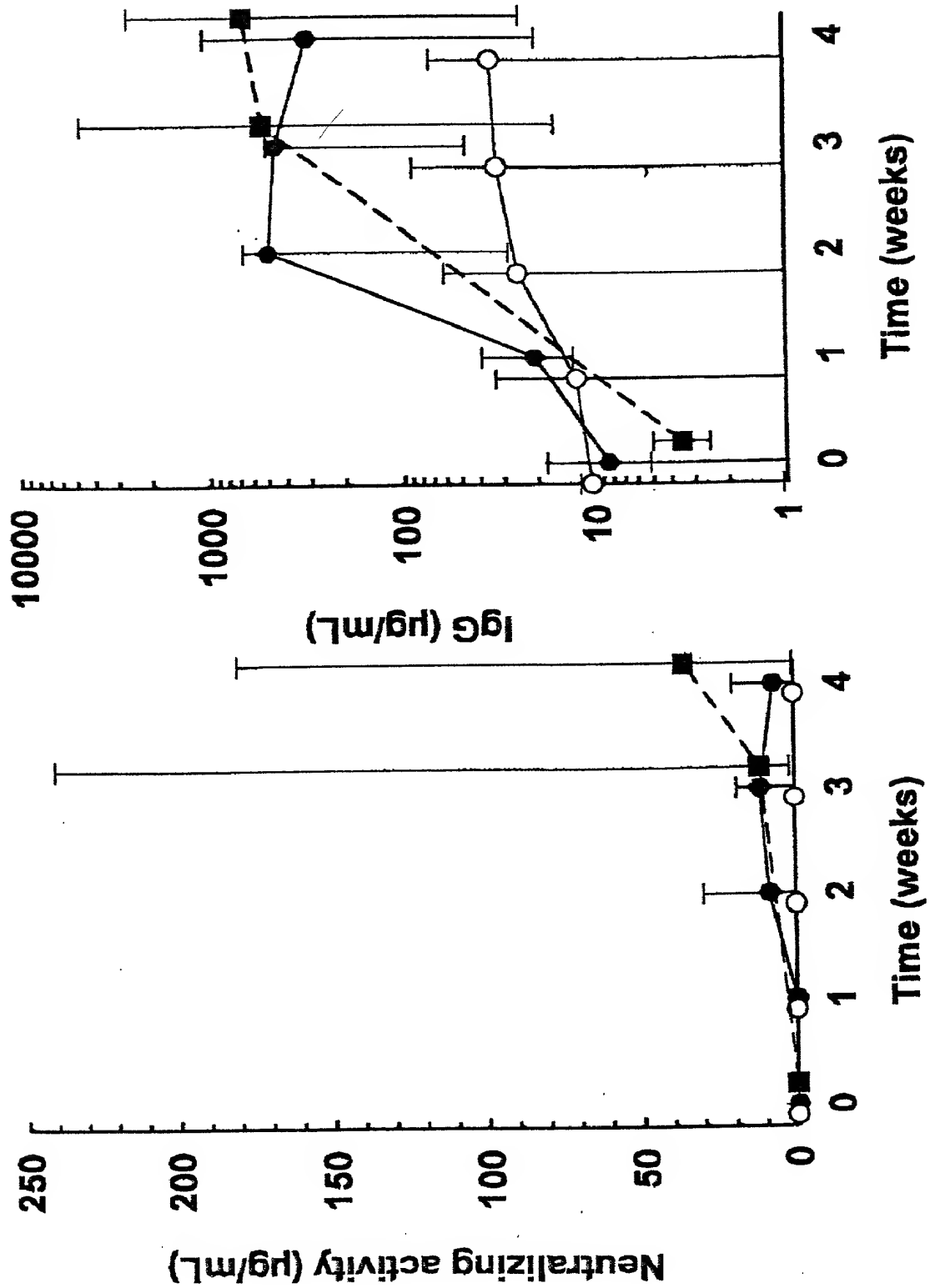


Figure 4

Figure 5



70222-04982/60

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Table 1: Alanine-to-wild-type" reversal variants of "charged-cluster-to-alanine" mutants of SakSTAR: Association constants ($K_A \times 10^7$ mol/L⁻¹) for the binding to insolubilized murine monoclonal antibodies (Mabs), and absorption (percent) of antibodies of immunized patient plasma

Variant	Exp. (mg/L)	Spec. Act. (kU/mg)	murine MAbs																SakSTAR patient plasma		
			Epitope I				Epitope II								Epitope III						
			17G11	26A2	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	1A10	Pool	Subpool B	Subpool C	
SakSTAR	130	130	22	13	2.9	7.8	11	38	7.4	19	7.7	2.4	4.0	14	3.4	2.9	0.6	95	95	95	
SakSTAR(K35A,E38A)	97	97	15	22	4.2	11	7.9	110	10	15	12	2.2	<0.1	<0.1	<0.1	1.0	1.0	93	91	94	
SakSTAR(K74A,E75A,R77A)	110	110	11	<0.1	<0.1	<0.1	<0.1	150	17	28	14	3.3	2.4	11	4.0	2.1	0.9	55	43	95	
SakSTAR(K35A,E38A,K74A,E75A,R77A)	50	50	11	<0.1	<0.1	<0.1	<0.1	110	36	26	15	2.0	<0.1	<0.1	<0.1	1.5	1.2	52	41	92	
SakSTAR(E38A,K74A,E75A,R77A)	43	43	11	<0.1	<0.2	<0.1	<0.1	140	39	26	15	2.1	<0.1	3.2	3.7	1.6	1.1	50	44	95	
SakSTAR(K35A,K74A,E75A,R77A)	56	56	9.2	<0.1	0.15	<0.1	<0.1	52	14	29	8.8	2.3	<0.1	1.8	<0.1	1.8	0.8	46	43	95	
SakSTAR(K35A,E38A,E75A,R77A)	44	44	11	0.3	0.1	0.2	<0.1	75	9.8	12	7.3	1.6	<0.1	<0.1	<0.1	0.53	0.64	92	87		
SakSTAR(K35A,E38A,K74A,R77A)	41	41	8.8	2.9	<0.1	2.0	0.33	110	29	31	10	2.0	<0.1	<0.1	<0.1	0.63	0.74	56	50	93	
SakSTAR(K35A,E38A,K74A,E75A)	19	19	13	<0.1	0.1	<0.1	<0.1	180	41	37	15	1.6	<0.1	<0.1	<0.1	1.2	0.45	48	41	92	
SakSTAR(E38A,E75A,R77A)	88	88	11	0.6	0.15	0.4	0.3	79	12	15	10	2.0	<0.1	2.6	4.7	1.1	0.81	95	88	95	
SakSTAR(E38A,E75A)	66	66	16	0.3	<0.1	<0.1	0.9	56	11	13	8.9	2.0	<0.1	20	4.8	1.3	1.6	91	90	95	
SakSTAR(K35A,E75A,R77A)	68	68	9.2	<0.1	<0.1	<0.1	<0.1	60	7.0	13	11	3.3	<0.1	1.5	<0.1	0.8	1.1	88	89	95	
SakSTAR(K35A,E75A)	150	150	17	0.12	<0.1	0.16	0.14	40	7.2	13	9.2	4.2	<0.1	1.8	<0.1	1.4	1.5	94	93	95	
SakSTAR(K74A)	100	100	12	7.6	0.17	4.4	2.1	55	15	33	14	3.6	2.9	14	4.9	3.4	1.2	59	45	95	
SakSTAR(E75A)	140	140	13	1.2	<0.1	<0.1	<0.1	46	8.5	14	12	3.4	4.5	18	5.0	1.2	2.1	95	93	95	
SakSTAR(K74A,E75A,R77A,E80A,D82A)	50	50	14	<0.1	<0.1	<0.1	<0.1	180	19	33	19	3.7	<0.1	<0.1	<0.1	<0.1	1.2	49	29	89	
SakSTAR(E80A,D82A)	130	130	7.3	12	2.1	6.5	5.9	79	6.1	8.4	7.8	1.9	<0.1	<0.1	<0.1	<0.1	0.44	89	83	92	
SakSTAR(E80A)	160	160	13	13	3.3	7.9	10	35	7.4	17	8.6	2.1	<0.1	16	3.6	<0.1	1.7	94	93	95	
SakSTAR(D82A)	160	160	17	12	4.8	7.3	11	31	7.8	17	12	2.7	<0.1	0.18	<0.1	<0.1	2.3	95	93		
SakSTAR(E75A,D82A)	170	170	20	15	3.1	6.6	7.2	69	8.1	15	14	4.9	0.17	0.7	0.5	0.1	1.4	95	95	95	

Apparent association constants ≥ 10 -fold lower than those of wild-type SakSTAR are represented in bold type; Spec. Act. $\geq 100,000$ HU/mg represented in bold type; $\leq 60\%$ absorption represented in bold type.

Table 2: Baseline characteristics and treatment outcome of the patients with peripheral arterial occlusion treated with SakSTAR, SakSTAR(K74A) or SakSTAR(K74A,E75A,R77A)⁴⁶

Compound Patient Id.	Gender	Age (yrs)	Clinical ischemia	Locus of occlusion	Age of occlusion (days)	Length of occlusion (cm)	Recanalization by thrombolysis	Total dose of thrombolytic agent (mg)	Total duration of infusion (hrs)	Additional therapy
SakSTAR										
MEE	F	67	Rest pain	Left SFA	30	8	complete	7.0	5.0	PTA
FOR	M	68	Claudication	Left IA (stent)	14	18	complete	6.5	4.5	PTA + stent
DAN	M	73	Claudication	Right SFA	30	6	complete	7.5	5.5	PTA
BER	F	63	Rest pain	Left FT graft	18	55	complete	18	28	PTA
DAM	F	43	Acute	Left brachial and radial artery	2	7	complete	19	17	PTA + stent
TOR	M	68	Claudication	Right SFA (popliteal aneurysm)	50	12	complete	6.0	4.0	PTA + femoropopliteal bypass graft
CLA	M	74	Acute	Left PA	1.5	20	complete	9.0	7.0	-
MAN	M	65	Acute	Left EIA (stent)	4	20	complete	6.5	4.5	(amputation left digit V)
MAT	M	64	Subacute	Right FP graft	3	45	complete	8.0	6.0	(-)
Mean ± SEM		65 ± 3.0			17 ± 5.6	21 ± 5.8		9.7 ± 1.7	9.1 ± 2.7	
SakSTAR(K74A)										
LIE	M	70	Subacute	Right FF graft	10	48	complete	11	9.0	PTA
ENG	M	50	Claudication	Right SFA	28	10	complete	12	10	PTA
COX	F	48	Claudication	Right PA graft	25	7	partial	15	15	PTA
MAN	F	68	Claudication	Right SFA	≥120	9	complete	9.0	7.0	PTA
VHE	M	47	Acute	Right IF graft	10	54	complete	18	16	Surgical graft revision
MUL	F	51	Acute	Right IF and FP graft	1	63	complete	16	20	PTA
BUR	F	67	Rest pain	Right TF trunc	9.0	38	partial	18	21	-
NUJ	F	60	Rest pain	Left AF graft	23	78	complete	15	21	-
POB*	M	49	Subacute	Right TF trunc	2	30	partial	6.0	4.0	rt-PA, surgical graft lengthening
VBE	M	39	Subacute	Right BA (embolism)	20	28	complete	18	23	Stent right SC artery, first rib resection
SME	F	50	Subacute	TF trunc	18	32	complete	21	19	None
WOL	M	67	Subacute	Right PA	4	25	complete	16	22	-
Mean ± SEM		56 ± 3.0			23 ± 9.2	35 ± 6.4		15 ± 1.2	16 ± 1.9	
SakSTAR(K74A,E75A,R77A)										
JAC	F	65	Acute	Right BA and UA	0.3	5	complete	14	12	-
MAE	M	74	Rest pain	Left SFA	10	50	complete	9.0	7.0	PTA
CRA	F	52	Claudication	Right IA and FA artery	14	28	complete	25	23	PTA + stent
VDB	M	68	Claudication	Left SFA	90	12	complete	9.0	7.0	PTA
DUN	M	71	Subacute	Left SFA	14	6	complete	9.0	7.0	PTA
DEL	M	59	Acute	Right FT graft	3	42	complete	9.0	7.0	PTA
Mean ± SEM		65 ± 3.3			22 ± 14	24 ± 7.8		13 ± 2.6	11 ± 2.6	

AF: aortofemoral; BA: brachial artery; CIA: common iliac artery; FF: femorofibular; FP: femoropopliteal; FT: femorotibial; IA: iliac artery; IF: iliofemoral; IP: popliteal artery; PTA: percutaneous transluminal angioplasty; SFA: superficial femoral artery; TF: tibiofibular; UA: ulnar artery.

*Previous treatment with SakSTAR in 1994

Table 3: Alanine-substitution variants of SakSTAR: Association constants ($K_A \times 10^7 \text{ mol/L}^{-1}$) for binding to insolubilized murine monoclonal antibodies (Mab) and absorption (percent) of antibodies of immunized patient plasma

Variant	Exp. (mg/L)	Spec. Act. (kU/mg)	murine MAbs														Pool	SakSTAR patient plasma		
			Epitope cluster I				Epitope cluster II								Epitope cluster III					
			17G11	26A2	30A2	28I2	3G10	18F12	14H5	28H4	32B2	7H10	7H11	25E1	40C8	24C4		1A10	Subpool B	Subpool C
SakSTAR		120	9.3	13	2.9	7.8	11	38	7.4	19	7.7	2.4	4.0	14	5.4	2.9	95	95	95	
			16.4	17	7.4	19	35	18	25	>18	>14	1.1	0.6	11	16	6.3	2.0]			
SakSTAR(S34G,G36R,H43R)		120	10	14	3.3	7.5	11	<0.1	<0.1	<0.1	20	2.7	<0.1	<0.1	<0.1	0.15	1.7	87	76	75
SakSTAR(F4A)	LE																			
SakSTAR(D5A,K6A)	150		11.3	21	12	9.2	9.7	12	23	17	10	0.4	1.3	3.9	0.8	4.5	3.8]	95	95	95
SakSTAR(K8A,K10A)	24		11.8	16	5.1	29	15	22	16	26	18	1.0	0.93	11	1.1	18	0.75]	95	95	95
SakSTAR(Y9A)	24	78	22	49	6.6	2.3	16	44	8.4	20	14	2.6	2.4	16	9.6	1.1	0.5	96	95	95
SakSTAR(K11A,D13A,D14A)	LE																			
SakSTAR(D13A)	6	46	2.4	6.1	2.0	3.7	3.4	11	1.9	4.5	4.4	8.7	1.5	2.4	1.1	3.6	<0.1	95	94	95
SakSTAR(D14A)	14	30	5.1	13	4.0	6.6	3.1	38	7.7	12	15	2.2	2.7	6.0	3.2	5.6	<0.1	95	94	95
SakSTAR(S16A)	41	160	8.1	16	4.5	8.4	9.0	15	6.1	13	21	3.6	4.0	8.0	4.3	2.5	0.5	95	95	95
SakSTAR(Y17A,F18A)	27	30	13	22	3.3	10	9.5	21	4.6	6.7	12	2.5	1.2	18	6.5	3.4	<0.1	95	95	95
SakSTAR(E19A,P20A)	36	9	11	19	3.3	9.2	12	15	6.1	11	16	1.0	1.1	15	5.1	3.3	<0.1	93	93	95
SakSTAR(T21A)	54	170	4.8	15	2.4	8.7	9.6	32	11	24	18	1.3	1.8	9.6	2.9	5.6	0.6	95	95	95
SakSTAR(P23A)	41	67	14	31	4.4	14	22	41	5.3	37	13	10	4.0	11	7.6	3.1	1.9	91	95	95
SakSTAR(Y24A)	10	40	17	33	4.3	13	11	33	4.3	7.0	12	4.0	0.4	14	6.8	4.8	<0.1	95	95	95
SakSTAR(L25A)	LE																			
SakSTAR(M26A)	LE																			
SakSTAR(V27A)	62	50	3.3	15	1.6	7.8	7.4	12	2.9	3.7	33	2.0	1.3	5.9	2.8	4.2	1.2	95	95	95
SakSTAR(N28A)	90	<5	5.8	29	2.1	7.0	5.5	27	10	20	2.5	2.1	2.1	5.6	2.1	2.1	0.7	95	95	95
SakSTAR(N28A,V29A)	32	45	18	30	2.5	20	18	20	14	20	24	2.7	3.3	20	1.1	5.0	2.0	93	95	95
SakSTAR(T30A)	52	140	7.4	13	2.1	7.0	6.1	7.6	3.4	5.1	13	3.3	5.6	12	3.4	5.4	0.8	94	95	95
SakSTAR(V32A)	78	45	10	9.6	2.4	6.2	7.8	56	17	12	14	1.4	<0.1	<0.1	<0.1	<0.1	2.2	90	93	95
SakSTAR(D33A,K35A)	130	130	12.1	19	14	14	19	15	24	32	10	5.3	1.4	5.1	3.8	5.1	3.0]	95	95	95
SakSTAR(S34A)	29	110	17	24	4.6	9.5	11	28	11	22	15	2.9	3.1	8.8	3.8	2.0	0.2	95	95	93

Variant	Exp. (mg/L)	Spec. Act (kU/mg)	in vitro MAAs										Epitope cluster I				Epitope cluster II				Epitope cluster III				SakSTAR patient plasma	
			17G11 26A2					30A2 2B12 3G10					18F12 14H5 28H4 32B2 7F10				24C4				24C4		Pool	Subpool B	Subpool C	
			7G11	26A2	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	1A10									
SakSTAR(K35A)		230	6.4	14	3.3	8.0	7.4	31	11	12	11	2.6	<0.1	1.3	0.2	1.7	0.8	91	88	95						
SakSTAR(K35A,E38A)		97	15	22	4.2	11	7.9	110	10	15	12	2.2	<0.1	<0.1	<0.1	1.0	1.0	93	91	94						
SakSTAR(G36A)	14	72	3.5	9.8	1.5	5.7	6.5	52	4.2	17	9.2	1.4	<0.1	<0.1	0.9	5.0	1.0	86	83	78						
SakSTAR(N37A)	40	110	5.6	31	3.0	10	11	20	4.1	14	10	2.9	1.4	5.3	3.5	3.6	0.8	95	95	95						
SakSTAR(L39A,L40A)	8	<5	14	15	3.1	5.1	8.0	27	16	6.3	12	2.7	1.2	5.4	3.2	2.1	0.9	93	93	95						
SakSTAR(S41A,P42A)	24	48	10	25	4.1	13	12	11	3.0	1.9	27	2.7	3.2	1.5	4.8	3.6	1.1	95	95	95						
SakSTAR(H43A)	37	69	15	28	9.7	18	7.6	<0.1	<0.1	<0.1	9.1	1.5	2.0	23	7.8	7.2	1.6	95	95	95						
SakSTAR(H43A,Y44A)	13	<5	10	22	3.7	17	1.5	<0.1	<0.1	<0.1	1.4	3.0	2.3	11	5.2	2.1	0.1	95	95	95						
SakSTAR(V45A)	19	<5	16	5.6	1.4	4.8	6.3	2.2	0.2	1.7	32	2.6	2.1	8.3	1.1	2.8	1.6	91	92	95						
SakSTAR(E46A,K50A)	LE																									
SakSTAR(F47A)	6	<5	<0.1	4.0	1.0	3.9	3.4	5.7	2.7	2.8	8.5	1.7	0.9	8.8	3.0	3.0	0.9	90	82	97						
SakSTAR(H49A)	2	43	2.7	27	7.8	23	22	35	4.4	11	6.2	1.1	2.0	5.7	2.0	1.7	0.6	95	95	95						
SakSTAR(K50A)	15	42	<0.1	13	2.9	7.8	8.7	46	8.3	12	2.2	0.5	2.8	6.4	4.0	2.3	0.6	95	94	95						
SakSTAR(T53A,T54A)	14	68	0.9	19	2.7	7.6	7.8	41	6.7	12	15	1.5	1.9	5.1	2.3	1.0	0.6	93	94	95						
SakSTAR(L55A)	LE																									
SakSTAR(T56A)	17	150	5.5	15	3.2	12	13	56	5.3	12	11	2.0	3.5	6.1	2.7	4.3	1.2	94	92	95						
SakSTAR(K57A,E58A,K59A)		94	14	8.7	6.0	7.3	27	16	14	6.7	5.6	0.52	0.36	1.7	0.42	1.0	1.1									
SakSTAR(I60A)	11	96	12	20	2.9	11	13	27	6.4	25	2.7	1.5	0.7	5.8	2.9	1.7	1.0	95	95	95						
SakSTAR(E61A,E65A)		80	[9.5	>10	8.8	21	29	>11	>16	6.6	>2	4.6	0.5	4.6	2.0	5.9	1.5									
SakSTAR(Y62A,Y63A)	24	<5	<0.1	4.3	0.3	2.1	1.9	11	2.2	3.1	8.4	1.7	0.6	9.2	3.6	3.8	0.7	89	83	95						
SakSTAR(Y63A)	4	<5	<0.1	18	3.7	9.6	13	17	5.3	3.3	15	1.1	2.2	5.3	4.3	1.0	3.7	89	82	95						
SakSTAR(V64A)	14	48	14	16	2.9	6.3	7.8	15	15	21	21	2.6	1.6	7.6	5.6	2.8	0.7	94	92	95						
SakSTAR(E65A)	25	97	53	20	4.4	12	7.0	10	5.6																	

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Variant	Exp. (mg/L)	Spec. Act. (KU/mg)	murine MAbs																SakSTAR patient plasma	
			Epitope cluster I				Epitope cluster II				Epitope cluster III				Pool	Subpool B	Subpool C			
			17G11	26A2	30A2	2B12	3G10	18P12	14H5	28H4	23D2	7F10	7H11	23E1				40C8	24C4	1A10
SakSTAR(Y73A)	20	<5	9.5	<0.1	<0.1	<0.1	4.8	3.3	7.3	1.1	8.9	2.0	0.5	9.0	2.5	1.4	0.8	44	33	
SakSTAR(Y73A,K74A)	24	<5	18	<0.1	<0.1	<0.1	19	6.7	23	9.9	3.2	2.7	13	4.0	1.6	1.1	47	28	87	
SakSTAR(K74A)	80	69	4.4	2.7	0.2	2.2	1.1	17	5.2	14	7.6	2.2	2.0	6.8	3.3	1.8	0.9	64	58	
SakSTAR(K74A,L75A,R77A)		68	9.2	<0.1	<0.1	<0.1	<0.1	60	7.0	13	11	3.3	<0.1	1.5	<0.1	0.8	1.1	88	89	
SakSTAR(K74A,R77A)	34	41	3.5	1.8	0.2	1.5	0.4	20	2.4	10	2.1	1.8	1.7	2.3	2.2	1.2	0.7	74	60	
SakSTAR(E75A)		140	13	1.2	<0.1	<0.1	<0.1	46	8.5	14	12	3.4	4.5	18	5.0	1.2	2.1	95	93	
SakSTAR(F76A)	9	90	20	9.6	1.0	2.7	3.9	13	6.2	20	15	1.7	0.3	5.9	2.1	1.2	1.0	94	92	
SakSTAR(V78A,V79A)	23	68	12	23	4.0	10	17	21	18	34	28	2.3	1.6	4.7	<0.1	0.5	1.7	93	93	
SakSTAR(E80A)		160	13	13	3.3	7.9	10	35	7.4	17	8.6	2.1	<0.1	1.6	3.6	<0.1	1.7	94	93	
SakSTAR(E80A,D82A)		130	7.3	12	2.1	6.5	5.9	79	6.1	8.4	7.8	1.9	<0.1	<0.1	<0.1	0.4	0.4	89	83	
SakSTAR(L81A)	23	28	12	33	1.6	40	11	52	11	17	17	3.9	1.4	5.2	7.1	4.6	1.5	88	95	
SakSTAR(D82A)		160	17	12	4.8	7.3	11	31	7.8	17	12	2.7	<0.1	0.2	<0.1	<0.1	2.3	95	93	
SakSTAR(D82A,S84A)	72	130	8.3	14	2.6	8.1	8.5	23	3.8	12	11	1.7	<0.1	<0.1	1.4	<0.1	1.0	91	91	
SakSTAR(S84A)	1226	89	8.0	16	3.8	8.6	10	90	8.3	11	3.6	1.8	2.2	1.6	3.0	3.5	0.5	95	95	
SakSTAR(K86A,E88A)		73	17.2	1.4	3.7	6.0	4.6	5.7	4.9	7.7	15	4.4	<0.1	5.4	0.80	1.9	0.13	95	95	
SakSTAR(I87A)	18	98	6.7	23	2.8	8.6	9.1	10	3.6	11	7.4	2.7	1.1	7.8	3.4	4.5	1.0	95	95	
SakSTAR(V89A)	20	87	4.6	11	2.6	6.6	2.2	28	7.2	7.3	3.0	1.3	1.2	5.1	2.9	3.1	0.83	95	95	
SakSTAR(T90A)	78	120	6.0	12	0.9	3.7	3.1	20	4.8	7.2	<0.1	<0.1	2.1	6.6	2.6	2.1	0.5	95	95	
SakSTAR(Y91A)	5	53	6.0	16	3.0	7.0	13	28	8.2	16	0.6	2.1	1.4	3.7	1.6	1.6	0.2	95	95	
SakSTAR(Y92A)	16	120	16	23	4.1	13	12	29	7.3	18	<0.1	1.7	4.4	10	3.9	5.9	1.1	95	95	
SakSTAR(E93A,K94A)		97	18.2	19	13	30	24	18	11	>10	9.0	0.88	1.4	11	2.4	7.0	2.11	95	95	
SakSTAR(K94A,N95A,K97A)	32	8	NT															95	94	
SakSTAR(N95A)	25	260	10	18	4.0	10	11	50	13	14	4.9	2.3	3.7	7.3	4.7	2.9	0.8	95	95	
SakSTAR(K96A,K97A,K98A)		47	7.8	41	23	37	90	>16	9.1	19	16	0.41	0.58	17	1.2	1.3	0.301			
SakSTAR(E99A)	24	42	7.4	15	4.0	8.4	8.9	22	2.7	4.7	<0.1	<0.1	2.1	6.2	7.3	1.4	0.8	92	91	
SakSTAR(E99A,I100A)	LE																			

Table 3 - cont'd: Alanine-substitution variants of SakSTAR: Association constants ($K_A \times 10^7 \text{ mol/L}^{-1}$) for binding to insolubilized murine monoclonal antibodies (Mab) and absorption (percent) of antibodies of immunized patient plasma

Variant	Exp. (mg/L)	Spec. Act. (kU/mg)	murine MAbs															SakSTAR patient plasma		
			Epitope cluster I					Epitope cluster II					Epitope cluster III					Pool	Subpool B	Subpool C
			17G11	26A2	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	1A10			
SakSTAR(T101A)	23	85	4.6	11	2.1	6.6	7.3	30	2.8	16	0.7	1.0	1.3	5.4	2.4	2.9	0.8	95	95	95
SakSTAR(K102A)	12	89	4.9	12	3.7	6.5	6.3	30	3.1	15	8.2	6.1	0.8	4.2	1.9	10	0.6	95	93	95
SakSTAR(S103A)	67	210	9.0	16	5.0	9.4	9.1	19	5.9	13	13	3.6	3.9	8.3	4.7	2.8	0.9	94	95	95
SakSTAR(F104A)	14	55	5.8	19	4.8	14	27	7.3	5.0	14	4.8	<0.1	0.4	7.6	3.4	1.1	1.3	95	93	95
SakSTAR(I106A)	2	93	2.3	13	3.0	7.4	6.7	5.5	5.2	17	11	1.4	1.8	3.1	1.8	1.2	0.5	95	95	95
SakSTAR(T107A)	32	130	5.2	15	3.4	9.8	10	32	8.7	4.7	14	1.9	3.1	6.3	3.2	5.0	0.8	94	94	95
SakSTAR(E108A,K109A)		170	[1.6	5.1	7.2	19	5.1	28	15	21	21	1.2	0.43	6.9	1.4	10	1.9			
SakSTAR(F111A)	5	49	3.7	16	3.8	13	22	21	8.4	12	3.1	0.8	2.8	2.9	1.5	1.5	0.9	95	95	95
SakSTAR(V112A,V113A)	64	130	4.2	16	3.9	10	12	34	5.8	13	8.0	0.3	1.5	4.3	2.3	3.0	0.8	95	95	95
SakSTAR(D115A,S117A)	80	54	3.3	14	4.1	15	15	17	3.4	19	0.7	<0.1	1.5	4.8	2.6	1.3	0.9	95	95	95
SakSTAR(D115A,E118A,H119A)		32	[2.5	32	3.4	21	8.7	13	9.9	23	9.3	1.2	1.0	24	2.1	9.0	1.8			
SakSTAR(L116A,S117A)	25	<5	4.4	35	3.6	33	42	160	29	220	<0.1	<0.1	0.5	4.1	4.9	3.5	1.6	94	95	95
SakSTAR(H119A,K121A)		130	[8.0	24	11	26	29	25	14	29	12	0.52	1.2	11	2.9	20	1.2			
SakSTAR(I120A)	26	75	23	26	5.1	17	16	30	9.8	25	9.0	6.9	3.0	15	5.1	5.2	1.0	93	95	95
SakSTAR(N122A)	5	19	NT															95	93	95
SakSTAR(F125A)	3	<10	2.8	18	4.7	11	18	17	3.2	6.0	1.9	<0.1	0.3	5.3	2.1	0.9	1.6	93	90	95
SakSTAR(N126V)	11	51	7.6	13	2.0	12	13	30	5.8	200	9.8	2.5	1.8	8.0	4.2	6.5	0.7	95	95	95
SakSTAR(L127A)	11	54	8.9	6.7	1.8	5.0	6.6	25	4.9	14	8.4	1.5	0.9	1.9	0.9	2.5	1.8	93	94	95
SakSTAR(I128A)	10	20	16	25	4.8	15	14	38	2.6	4.3	8.2	2.9	2.5	2.0	4.2	6.7	0.9	95	93	95
SakSTAR(T129A)	44	190	5.3	15	2.3	14	24	21	13	15	4.2	2.3	0.7	10	3.3	1.3	1.0	95	95	95
SakSTAR(K130A)	130	280	5.1	12	3.2	6.4	3.5	22	6.7	11	15	2.7	<0.1	<0.1	4.1	0.9	0.6	92	74	71
SakSTAR(V131A)	130	70	6.5	17	2.9	11	13	19	14	19	29	3.4	1.9	13	5.3	8.6	0.9	95	95	95
SakSTAR(V132A)	100	130	4.2	15	2.6	9.2	11	33	12	30	19	2.1	2.1	3.6	<0.1	2.6	0.4	95	95	95
SakSTAR(I133A)	3	99	9.4	15	1.9	7.8	7.8	24	6.0	9.1	8.6	1.4	0.56	6.4	1.6	1.6	0.9	95	95	95
SakSTAR(E134A,K135A,K136A)		74	[22	21	6.7	25	25	>18	>25	>15	>12	1.7	0.2	11	0.94	6.0	2.6]			
SakSTAR(K135A)	54	410	5.2	12	11	7.9	11	20	11	11	3.8	2.0	1.6	6.9	3.7	1.9	0.9	95	95	95
SakSTAR(K136A)		180	7.6	18	5.6	12	13	54	5.3	12	16	3.2	3.5	8.6	3.9	1.8	0.5	91	83	95

LE: expression level below 3 mg/L

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Table 4: Mutagenesis of S34, G36 and H43: Association constants ($K_A \times 10^7$ mol/L⁻¹) for binding to insolubilized murine monoclonal antibodies (Mab) and absorption (percent) of antibodies of immunized patient plasma

Variant	Exp. (mg/L)	Spec. Act (kU/mg)	Epitope cluster I					Epitope cluster II					Epitope cluster III					SakSTAR patient plasma		
			17G11	26A2	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	1A10	Pool	Subpool B	Subpool C
SakSTAR		120	9.3	13	2.9	7.8	11	38	7.4	19	7.7	2.4	4.0	1.4	3.4	2.9	0.6	95	95	95
SakSTAR(G34G,G36R,H43R)		120	10	14	3.3	7.5	11	<0.1	<0.1	<0.1	20	2.7	<0.1	<0.1	<0.1	0.15	1.7	87	76	75
SakSTAR(G34A)	29	110	17	24	4.6	9.5	11	28	11	22	15	2.9	3.1	8.8	3.8	2.0	0.2	95	95	93
SakSTAR(G36A)	14	72	3.5	9.8	1.5	5.7	6.5	52	4.2	17	9.2	1.4	<0.1	<0.1	0.9	5.0	1.0	86	83	78
SakSTAR(G36E)	12	66	3.1	8.7	1.4	4.8	4.7	12	2.8	6.1	7.6	1.0	<0.1	<0.1	<0.1	3.4	1.1	89	83	72
SakSTAR(G36K)	34	88	9.9	23	3.1	8.3	9.8	21	3.9	13	15	3.0	<0.1	<0.1	<0.1	2.6	1.2	88	80	69
SakSTAR(G36L)	15	92	3.6	11	1.8	6.1	6.1	16	1.4	6.4	12	1.3	<0.1	<0.1	1.0	0.3	0.5	86	80	75
SakSTAR(G36N)	10	91	8.7	10	1.6	6.1	6.2	33	3.3	7.8	7.9	1.8	<0.1	<0.1	1.0	0.3	0.5	86	80	75
SakSTAR(G36Q)	21	92	10	12	1.8	6.7	6.5	23	3.8	7.5	7.3	1.5	<0.1	<0.1	<0.1	0.1	0.4	87	84	73
SakSTAR(G36R)	45	100	11	2.4	3.3	10	10	27	4.6	14	20	3.4	<0.1	<0.1	<0.1	3.1	1.2	89	81	70
SakSTAR(H43A)	37	69	15	28	9.7	18	7.6	<0.1	<0.1	<0.1	9.1	1.5	2.0	23	7.8	7.2	1.6	95	95	95
SakSTAR(H43R)	43	120	15	11	2.7	7.6	11	<0.1	<0.1	<0.1	13	6.4	0.7	18	6.7	5.7	1.4	95	95	95
SakSTAR(S34G,G36R)	45	90	3.1	12	2.3	4.8	4.2	13	8.3	24	9.1	1.9	<0.1	<0.1	<0.1	<0.1	0.6	92	83	69
SakSTAR(S34G,G36R,H43R,K74A)	1	12	12	4.7	3.8	7.4	9.4	<0.1	0.1	0.6	25	2.3	<0.1	<0.1	0.5	0.8	1.7	67	56	83
SakSTAR(S34G,G36R,K74A)	15	26	4.0	2.1	<0.1	1.8	0.6	10	2.2	15	13	1.8	<0.1	<0.1	<0.1	0.3	2.2	59	28	68
SakSTAR(K35G,G36R,H43D)	32	6	1.8	1.5	1.6	2.0	8.9	<0.1	<0.1	<0.1	7.1	1.3	<0.1	<0.1	<0.1	<0.1	0.9	82	75	72
SakSTAR(G36R,K74A)	40	35	19	7.0	0.2	4.3	2.0	53	27	28	19	4.4	<0.1	<0.1	<0.1	1.2	1.0	48	33	58
SakSTAR(G36R,K74R)	68	150	4.7	17	3.8	11	8.0	16	6.0	6.4	3.0	1.6	<0.1	<0.1	<0.1	0.2	0.8	81	54	73
SakSTAR(G36R,K74A,N95A)	11	25	6.1	2.9	2.9	2.4	<0.1	31	5.7	12	5.3	4.1	<0.1	<0.1	<0.1	<0.1	0.9	53	32	63
SakSTAR(G36R,K74A,K135R)	20	33	5.8	3.4	<0.1	1.7	0.7	26	16	14	17	1.2	<0.1	<0.1	<0.1	0.4	0.5	64	32	68
SakSTAR(G36R,K74R,K135R)	48	75	6.1	17	5.8	10	3.3	31	14	13	5.7	2.3	<0.1	<0.1	<0.1	0.3	0.8	77	49	68

Table 5: Mutagenesis of K35, Y73, K74, E80/D82, N95, K130, V132 and K135: Association constants ($K_A \times 10^7 \text{ mol/L}^{-1}$) for binding to insolubilized murine monoclonal antibodies (Mab) and absorption (percent) of antibodies of immunized patient plasma

Variant	Exp. (mg/L)	Spec. Act (kU/mg)	murine MAbs										Epitope cluster III						SakSTAR patient plasma		
			Epitope cluster I					Epitope cluster II					Epitope cluster III						Pool	Subpool B	Subpool C
			17G11	26A2	30AX2	28B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	23E1	40C8	24C4	1A10				
SakSTAR		120	9.3	13	2.9	7.8	11	38	1.4	19	7.7	2.4	4.0	14	5.4	2.9	0.6	95	95	95	
SakSTAR(S34G,G36R,H43R)		120	10	14	3.3	7.5	11	<0.1	<0.1	<0.1	20	2.7	<0.1	<0.1	<0.1	0.15	1.7	87	76	75	
SakSTAR(K35A)	230		6.4	14	3.3	8.0	7.4	31	11	12	11	2.6	<0.1	1.3	0.2	1.7	0.8	91	88	95	
SakSTAR(K35E)	75	160	4.8	10	0.6	2.7	2.7	21	5.7	9.1	4.8	1.0	<0.1	<0.1	<0.1	1.5	0.4	95	95	92	
SakSTAR(K35Q)	9	69	3.2	9.5	1.3	5.2	5.4	22	2.7	9.3	8.5	1.2	0.5	1.7	<0.1	1.9	1.0	95	95	95	
SakSTAR(Y73A)	20	<5	9.5	<0.1	<0.1	4.8	33	7.3	11	8.9	2.0	0.5	0.5	9.0	2.5	1.4	0.8	63	44	93	
SakSTAR(Y73F)	6	31	7.9	12	1.3	2.1	8.0	24	15	19	15	2.6	1.6	6.2	3.5	7.2	1.4	93	95	95	
SakSTAR(Y73H)	30	<5	7.3	1.5	<0.1	<0.1	1.4	20	7.5	17	42	4.3	3.5	6.8	5.9	9.7	1.3	76	65	95	
SakSTAR(Y73L)	33	<5	11	<0.1	<0.1	<0.1	<0.1	84	19	26	53	3.4	4.2	7.1	4.2	3.4	1.0	81	60	94	
SakSTAR(Y73S)	31	<5	7.0	3.6	0.59	0.6	3.0	21	8.3	14	21	3.2	2.2	6.7	1.3	1.3	0.7	86	69	95	
SakSTAR(Y73W)	6	27	4.8	9.0	4.6	4.6	11	8.4	4.5	11	8.0	2.7	2.9	5.0	3.0	3.8	1.3	73	53	93	
SakSTAR(K74A)	80	69	4.4	2.7	0.2	2.2	1.1	17	5.2	14	7.6	2.2	2.0	6.8	3.3	1.8	0.9	64	58	95	
SakSTAR(K74E)	12	<5	2.2	0.6	<0.1	0.7	0.1	14	1.8	7.5	9.3	1.2	2.0	3.0	1.2	0.6	1.0	83	43	90	
SakSTAR(K74N)	9	39	2.9	4.7	1.1	3.3	1.7	10	1.6	4.8	13	1.5	1.9	4.0	1.8	1.4	0.9	63	46	95	
SakSTAR(K74Q)	64	110	5.3	5.8	<0.1	2.5	1.1	24	5.9	12	5.4	1.1	2.0	6.2	2.3	2.0	0.4	70	62	94	
SakSTAR(K74R)	44	150	2.1	7.5	2.0	4.1	4.2	24	6.9	8.0	8.3	1.3	2.2	7.8	3.2	2.1	0.5	75	70	95	
SakSTAR(E80A,D82A)	130		7.3	12	2.1	6.5	5.9	79	6.1	8.4	7.8	1.9	<0.1	<0.1	<0.1	0.4		89	83	92	
SakSTAR(E80A)	160		13	13	3.3	7.9	10	35	7.4	17	8.6	2.1	<0.1	16	3.6	<0.1	1.7	94	93	95	
SakSTAR(D82A)	160		17	12	4.8	7.3	11	31	7.8	17	12	2.7	<0.1	0.2	<0.1	<0.1	2.3	95	93	95	
SakSTAR(N95A)	25	260	10	18	4.0	10	11	50	13	14	4.9	2.3	3.7	7.3	4.7	2.9	0.8	95	94	95	
SakSTAR(N95E)	12	79	2.8	8.5	1.3	5.2	5.4	17	2.7	10	5.2	1.1	0.5	4.0	1.7	1.8	0.6	95	92	95	
SakSTAR(N95G)	20	160	4.1	11	1.5	6.8	7.6	36	3.3	15	3.6	1.5	0.7	5.8	2.7	2.7	0.9	95	90	95	
SakSTAR(N95K)	54	180	9.5	14	3.2	9.0	11	51	5.0	18	4.8	2.5	1.6	8.3	2.9	4.8	1.1	95	95	95	
SakSTAR(N95R)	LE																				

Sak5TAR(I60A,K74A,N95A)

Table 6: Combination mutants of SakSTAR(K130T,K135R) with K35A, G36R, E65X,K74X and selected other amino acids

Variant	Exp. (mg/mL)	Spec. Act. (kU/mg)	murine MAbs										SakSTAR patient plasma				Code					
			Epitope cluster I			Epitope cluster II				Epitope cluster III			Pool 10	Subpool B	Subpool C	Pool 40						
			17G11	26A32	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	1A10					
SakSTAR(K130T,K135R)	15	280	3.7	10	1.6	7.2	8.0	13	7.7	4.5	3.7	1.6	<0.1	<0.1	2.4	0.4	0.6	89	60	73	90	SY2
SakSTAR(G36R,K130T,K135R)	26	220	7.2	18	2.8	11	14	17	3.9	8.9	5.1	1.6	<0.1	<0.1	<0.1	<0.1	1.1	79	65	69	-	SY3
SakSTAR(K74R,K130T,K135R)	18	310	7.3	27	5.9	16	18	17	3.7	11	5.1	1.5	<0.1	<0.1	3.2	0.7	0.9	76	49	69	78	SY4
SakSTAR(K74Q,K130T,K135R)	64	190	4.0	7.2	3.0	7.7	0.9	20	5.7	7.2	8.4	1.8	<0.1	<0.1	2.7	0.7	1.0	50	25	67	62	SY41
SakSTAR(G36R,K74R,K130T,K135R)	5	210	7.6	26	5.7	17	19	17	4.7	10	5.0	1.4	<0.1	<0.1	<0.1	<0.1	0.8	73	44	69	75	SY5
SakSTAR(G36R,K74Q,K130T,K135R)	88	120	5.5	7.3	0.8	11	6.4	35	11	7.1	6.1	2.6	<0.1	<0.1	<0.1	<0.1	0.8	51	25	63	54	SY42
SakSTAR(G36R,H43R,K74R,K130T,K135R)	29	160	5.6	8.8	2.2	10	10	<0.1	<0.1	<0.1	6.3	2.0	<0.1	<0.1	<0.1	<0.1	0.9	72	39	69	-	SY9
SakSTAR(G34Q,G36R,K74Q,K130T,K135R)	40	76	4.8	5.9	0.5	13	1.5	18	8.7	7.5	5.1	2.1	<0.1	<0.1	<0.1	<0.1	1.1	52	25	65	61	SY43
SakSTAR(E65A,K74Q,K130T,K135R)	46	170	11	9.0	2.3	11	13	19	66	12	5.7	2.3	<0.1	<0.1	3.5	1.2	0.6	45	16	77	55	SY48
SakSTAR(G36R,E65A,K74Q,K130T,K135R)	80	83	4.7	12	1.2	16	21	27	12	10	6.9	2.6	<0.1	<0.1	<0.1	<0.1	0.7	44	18	65	46	SY44
SakSTAR(G36R,E65A,K74A,K130A,K135R)	17	71	5.7	10	1.8	13	12	21	8.4	6.9	4.6	1.8	<0.1	<0.1	<0.1	<0.1	1.0	41	14	64	50	SY59
SakSTAR(E65A,A72S,K74Q,K130T,K135R)	60	96	5.6	6.0	2.0	3.6	4.9	21	8.6	8.8	2.8	2.5	<0.1	<0.1	3.5	1.7	1.1	51	13	66	56	SY51
SakSTAR(E65Q,K74Q,K130T,K135R)	40	150	6.7	18	2.1	15	16	9.0	3.1	4.1	6.3	2.3	<0.1	<0.1	3.8	0.9	0.6	53	29	67	65	SY49
SakSTAR(K74Q,K86A,K130T,K135R)	54	130	2.4	4.9	<0.1	7.4	3.8	19	8.7	7.6	4.7	1.9	<0.1	<0.1	3.5	1.3	1.5	56	32	69	61	SY55
SakSTAR(E65Q,T71S,K74Q,K130T,K135R)	32	210	6.2	13	1.8	10	13	11	3.9	5.0	6.8	2.3	<0.1	<0.1	3.1	1.1	0.8	49	21	64	59	SY65
SakSTAR(E65Q,K74Q,E75A,K130T,K135R)	36	46	7.7	<0.1	<0.1	<0.1	<0.1	10	3.9	3.2	7.4	2.6	<0.1	<0.1	4.6	1.3	0.6	43	15	62	55	SY66
SakSTAR(E65Q,K74Q,E75D,K130T,K135R)	35	67	7.0	<0.1	<0.1	<0.1	<0.1	13	5.4	4.9	6.6	2.5	<0.1	<0.1	3.2	1.3	1.0	49	29	63	57	SY67
SakSTAR(K74Q,K130T,K135R,K136A,+137A)	19	78	4.3	24	<0.1	2.7	5.6	20	9.6	7.5	5.6	2.2	<0.1	<0.1	1.7	1.1	1.2	37	12	57	50	SY68
SakSTAR(K74Q,K130A,K135R)	28	240	5.6	5.4	0.5	7.5	5.3	21	8.4	14	6.1	2.4	<0.1	<0.1	4.1	2.6	0.7	57	27	78	65	SY56
SakSTAR(E65Q,K74Q,K130A,K135R)	60	230	6.0	14	2.4	15	17	9.0	3.3	5.8	6.3	2.3	<0.1	<0.1	4.3	2.0	0.6	51	32	73	58	SY69
SakSTAR(K74Q,K130E,K135R)	46	300	4.1	4.4	0.8	6.6	3.8	18	8.5	7.8	5.2	2.1	<0.1	<0.1	2.4	0.7	0.9	55	29	64	59	SY57
SakSTAR(E65Q,K74Q,K130A,K135A)	88	170	5.3	8.9	1.7	7.7	11	16	3.4	5.8	6.2	2.4	<0.1	<0.1	3.5	2.4	0.7	55	27	79	55	SY70
SakSTAR(K74Q,K130E,Y132R,K135R)	68	170	4.5	4.9	0.4	6.0	5.2	9.0	4.9	4.3	5.6	2.4	<0.1	<0.1	<0.1	<0.1	0.6	51	20	63	56	SY58
SakSTAR(E65Q,K74Q,T90A,K130A,K135R)	36	170	6.2	13	1.8	12	14	7.3	2.5	3.8	<0.1	<0.1	<0.1	<0.1	4.1	1.9	0.5	51	27	69	57	SY71

Table 6 - cont'd: Combination mutants of SakSTAR(K130T,K135R) with K35A, G36R, E65X,K74X and selected other amino acids

Variant	Exp. mg/L	Spec. Act. (AU/mg)	murine K1A1s										Epitope cluster II				Epitope cluster III				SakSTAR patient plasma				Code
			Epitope cluster I					Epitope cluster II					Epitope cluster III				Epitope cluster III				Epitope cluster III				
			17G11	26A2	30A2	2B12	3G10	18R12	14H5	28H4	12B2	7F10	7H11	25E1	40C8	24C4	1A10	Post 10	Subpool B	Subpool C	Post 40				
SakSTAR(E65Q,K74Q,N95A,K130A,K135R)	40	220	6.1	14	1.9	1.7	15	9.0	3.3	3.8	6.3	2.3	<0.1	<0.1	4.1	2.2	0.5	52	29	74	58	58	58	SY72	
SakSTAR(E65Q,K74Q,E118A,K130A,K135R)	86	180	8.5	18	2.8	1.5	27	11	4.1	5.7	7.3	2.6	<0.1	<0.1	6.1	2.8	0.5	50	28	72	58	58	58	SY73	
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R)	33	190	7.8	18	2.4	7.7	21	20	3.9	6.1	8.6	2.3	<0.1	<0.1	5.8	2.5	0.5	48	27	74	58	58	58	SY74	
SakSTAR(N95A,K130A,K135R)	85	410	6.1	11	3.3	18	15	37	5.9	9.6	6.8	2.5	<0.1	<0.1	4.5	3.0	0.6	93	81	82	94	94	94	INT1	
SakSTAR(K35A,E65Q,K74Q,K130A,K135R)	29	110	NT															49	26	63	45	45	45	SY75	
SakSTAR(K35A,H43R,E65Q,K74Q,K130A,K135R)	14	14	NT															49	23	73	55	55	55	SY76	
SakSTAR(E65Q,K74Q,S103A,K130A,K135R)	32	60	6.7	15	2.6	14	16	8.0	2.7	3.9	6.3	2.3	<0.1	<0.1	4.6	1.6	0.6	55	27	75	61	61	61	SY77	
SakSTAR(T21A,K35A,E65Q,K74Q,K130A,K135R)	110	110	NT															50	26	72	50	50	50	SY78	
SakSTAR(T36A,E65Q,K74Q,K130T,K135R)	180	180	NT															52	31	61	55	55	55	SY79	
SakSTAR(K37A,E38A,E61A,K74Q,K130T,K135R)	120	120	NT															57	24	61	54	54	54	SY80	
SakSTAR(E65Q,K74Q,K109A,K130T,K135R)	40	210	7.3	15	2.1	12	12	14	2.5	4.0	5.8	2.3	<0.1	<0.1	3.4	1.8	0.7	50	22	68	51	51	51	SY81	
SakSTAR(E65Q,K74Q,E108A,K130T,K135R)	120	120																51	24	61	54	54	54	SY82	
SakSTAR(E65Q,K74Q,E108A,K109A,K130T,K135R)	62	180	9.3	13	1.4	13	17	17	3.0	4.1	6.8	2.5	<0.1	<0.1	3.7	2.6	0.5	55	21	67	50	50	50	SY83	
SakSTAR(E65Q,K74Q,K121A,K130T,K135R)	73	150	5.7	13	1.5	11	14	22	3.1	4.6	1.2	<0.1	<0.1	<0.1	3.5	1.8	0.9	61	25	69	57	57	57	SY85	
SakSTAR(E19A,E65Q,K74Q,K130T,K135R)	3	3	NT															51	27	62	56	56	56	SY86	
SakSTAR(E65Q,K74Q,D115A,K130T,K135R)	57	57	NT															52	25	62	56	56	56	SY87	
SakSTAR(G36R,E65A,K74Q,K130E,V132R,K135R)	48	60	7.6	9.9	1.4	11	14	42	19	17	4.3	1.0	<0.1	<0.1	<0.1	<0.1	0.9	44	17	70	44	44	44	SY60	
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R,+137A)	120	120																45	30	74	60	60	60	SY93	
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R,+137K)	1,400	1,400																37	16	70	54	54	54	SY94	

Association constants ≥ 10 -fold lower and antibody absorption ≤ 60 percent of wild-type SakSTAR are represented in bold type; $\geq 100,000$ HU/mg represented in bold type. NT: not tested.

Table 7: Combination mutants of SakSTAR(E80A,D82A,K130T,K135R) with K35A, G36R, E65X, K74X, and selected other amino acids

Variant	Exp. (mg/mL)	Spec. Act. (kU/mg)	murine MAbs																SakSTAR patient plasma				Code			
			Epitope cluster I						Epitope cluster II						Epitope cluster III				Pool T0		Subpool B			Subpool C		Pool 40
			17G11	26A2	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	JA1	0								
SakSTAR(E80A,D82A,K130T,K135R)	20	250	8.4	17	3.1	11	13	18	4.3	11	5.5	1.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	80	64	68	SY6				
SakSTAR(K74R,E80A,D82A,K130T,K135R)	4	220	5.3	31	2.8	18	11	89	8.4	21	5.9	1.8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	74	34	69	SY7				
SakSTAR(K74Q,E80A,D82A,K130T,K135R)	27	110	5.1	6.5	1.2	7.9	7.3	28	6.4	19	4.6	1.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	46	17	60	SY15				
SakSTAR(K35A,K74R,E80A,D82A,K130T,K135R)	70	160	5.9	5.7	6.4	2.7	18	19	16	8.2	9.0	1.8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	66	34	66	SY17				
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	22	140	5.4	50	2.9	49	21	31	11	37	3.4	1.8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	43	11	68	SY19				
SakSTAR(E65S,K74R,E80A,D82A,K130T,K135R)	3	110	3.2	12	4.6	44	15	19	5.0	15	6.6	1.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	35	12	60	SY20				
SakSTAR(E65T,K74R,E80A,D82A,K130T,K135R)	30	94	7.2	9.3	9.9	5.6	32	22	15	8.2	11	2.0	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	58	24	69	SY21				
SakSTAR(S34G,G36R,K74R,K130T,K135R)		250	5.6	54	2.9	38	25	36	6.5	18	5.5	1.9	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	75	33	68	SY10				
SakSTAR(E65A,K74R,E80A,D82A,K130T,K135R)	40	140	8.2	10	13	5.7	55	24	22	11	15	2.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	51	17	66	SY18				
SakSTAR(E65N,K74R,E80A,D82A,K130T,K135R)	88	120	8.5	12	11	7.0	36	43	13	12	7.9	2.4	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	60	29	67	SY23				
SakSTAR(E65Q,K74R,E80A,D82A,K130T,K135R)	55	140	9.0	16	16	9.9	59	18	4.5	4.3	10	2.5	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	54	22	63	SY22				
SakSTAR(K57A,E58A,E61A,E80A,D82A,K130T,K135R)	24	110	2.4	17	2.9	13	12	16	7.8	16	4.5	1.6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	75	64	62	SY13				
SakSTAR(E65A,A72S,K74R,E80A,D82A,K130T,K135R)	92	62	8.2	23	3.8	8.4	19	30	11	13	3.6	3.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	51	13	66	SY53				
SakSTAR(E65D,K74Q,E80A,D82A,K130T,K135R)	84	110	7.0	3.9	2.6	4.1	7.2	29	28	14	6.8	2.2	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	43	13	64	SY30				
SakSTAR(E65Q,K74Q,E80A,D82A,K130T,K135R)	54	120	5.1	16	3.2	14	3.7	16	20	2.4	5.7	1.7	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	43	21	64	SY47				
SakSTAR(K35A,E65D,K74Q,E80A,D82A,K130T,K135R)	56	140	5.1	6.8	2.6	9.5	1.7	28	17	14	8.4	2.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	35	8	58	SY46				

Table 7 - cont'd: Combination mutants of SakSTAR(E80A,D82A,K130T,K135R) with K35A, G36R, E65X, K74X, and selected other amino acids

Variant	Exp. (mg/mL)	Spec. Act. (kU/mg)	murine MAbs															SakSTAR patient plasma				Code
			Epitope cluster I					Epitope cluster II					Epitope cluster III					Pool 10	Subpool B	Subpool C	Pool 40	
			17G11	26A2	30A2	2B12	3G10	18F12	14H5	28H4	32B2	7F10	7H11	25E1	40C8	24C4	1A10					
SakSTAR(K74R,E80A,D82A,S103A,K130T,K135R)	32	160	4.9	22	5.8	14	4.3	26	6.9	7.4	5.0	1.7	<0.1	<0.1	<0.1	<0.1	67	32	69	70	SY24	
SakSTAR(K35A,E65D,K74R,E80A,D82A,E108A,K109A,K130T,K135R)	9.0	89	5.8	30	2.6	26	10	13	16	13	3.2	1.8	<0.1	<0.1	<0.1	<0.1	55	10	63	47	SY12	
SakSTAR(K35A,E65D,K74R,E80A,D82A,E108A,K130T,K135R)	20	91	6.4	20	5.0	15	3.9	22	17	7.4	2.4	1.9	<0.1	<0.1	<0.1	<0.1	44	8	70	53	SY32	
SakSTAR(E65D,K74R,E80A,D82A,E108A,K130T,K135R)	4	90	8.1	6.7	6.9	4.3	29	19	31	11	14	2.1	<0.1	<0.1	<0.1	<0.1	52	11	69	-	SY33	
SakSTAR(K35A,E65D,K74R,E80A,D82A,K109A,K130T,K135R)	42	84	5.5	18	5.3	14	1.6	18	12	7.7	10	1.7	<0.1	<0.1	<0.1	<0.1	43	6	61	50	SY36	
SakSTAR(E65D,K74R,E80A,D82A,K109A,K130T,K135R)	60	130	9.7	6.6	6.8	4.2	28	11	32	12	17	2.3	<0.1	<0.1	<0.1	<0.1	56	10	64	53	SY37	
SakSTAR(K35A,E65D,K74R,E80A,D82A,K130T,K135R,K136A)	28	81	4.5	12	3.3	11	1.7	22	13	7.6	4.9	1.6	<0.1	<0.1	<0.1	<0.1	40	14	52	40	SY34	
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R,K136A)	60	100	6.8	5.8	4.4	4.5	15	33	32	14	7.9	2.0	<0.1	<0.1	<0.1	<0.1	46	28	67	45	4	
SakSTAR(E65Q,K74Q,D82A,S84A,K130T,K135R)		170	NT														45	21	60	45	SY50N	
SakSTAR(K35A,E65D,K74R,E80A,D82A,K86A,K130T,K135T)	68	86	4.4	20	5.5	15	1.5	15	12	6.4	6.7	1.9	<0.1	<0.1	<0.1	<0.1	36	7	60	55	SY40	
SakSTAR(K35A,K74Q,E80A,D82A,K130T,K135R)	72	120	6.1	3.4	2.5	3.0	5.9	38	14	9.8	6.8	1.9	<0.1	<0.1	<0.1	<0.1	49	16	64	48	SY28	
SakSTAR(K35A,E65D,K74R,E80A,D82A,K130T,K135R)	54	190	8.1	7.5	6.9	5.5	25	37	34	14	7.7	2.3	<0.1	<0.1	<0.1	<0.1	56	28	68	55	SY29	
SakSTAR(K35A,E65D,K74R,E80A,D82A,V132R,K135R)	13	55	6.7	23	5.3	17	2.3	47	19	19	5.1	2.0	<0.1	<0.1	<0.1	<0.1	53	20	88	62	SY61	
SakSTAR(K35A,E65D,K74R,E80A,D82A,T129A,K135R)	13	61	7.0	13	5.1	31	12	27	12	11	6.7	2.5	<0.1	<0.1	<0.1	<0.1	56	18	79	60	SY62	
SakSTAR(K35A,E65D,K74R,E80A,D82A,T129A,K135A)	23	21	6.9	27	5.8	32	20	29	6.6	9.7	5.4	2.1	<0.1	<0.1	<0.1	<0.1	56	17	91	60	SY64	

Association constants ≥ 10 -fold lower and antibody absorption ≤ 60 percent of wild-type SakSTAR are represented in bold type; $\geq 100,000$ HU/mg represented in bold type. NT: not tested.

Table 8: SakSTAR variants with intact specific activity (≥ 100 kHU/mg) and ≤ 50 percent absorption of human antibodies elicited by treatment with wild-type SakSTAR

Variant	Spec. Act. (kU/mg)	SakSTAR patient plasma				Code
		Pool 10	Subpool B	Subpool C	Pool 40	
SakSTAR(K74Q,K130T,K135R)	190	50	25	67	62	SY41
SakSTAR(E65A,K74Q,K130T,K135R)	170	45	16	77	55	SY48
SakSTAR(E65Q,T71S,K74Q,K130T,K135R)	210	49	21	64	59	SY65
SakSTAR(E65Q,K74Q,E118A,K130A,K135R)	180	50	28	72	58	SY73
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R)	190	48	27	74	58	SY74
SakSTAR(K35A,E65Q,K74Q,K130A,K135R)	110	49	26	63	45	SY75
SakSTAR(E65Q,K74Q,K109A,K130T,K135R)	210	50	22	68	51	SY81
SakSTAR(K74Q,E80A,D82A,K130T,K135R)	110	46	17	60	48	SY15
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	140	43	11	68	57	SY19
SakSTAR(E65S,K74R,E80A,D82A,K130T,K135R)	110	35	12	60	-	SY20
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R,K136A)	100	46	28	67	45	SY35
SakSTAR(K35A,K74Q,E80A,D82A,K130T,K135R)	120	49	16	64	48	SY28
SakSTAR(E65D,K74Q,E80A,D82A,K130T,K135R)	110	43	13	64	42	SY30
SakSTAR(E65Q,K74Q,E80A,D82A,K130T,K135R)	120	43	21	64	42	SY47
SakSTAR(E65Q,K74Q,D82A,S84A,K130T,K135R)	170	45	21	60	45	SY50N
SakSTAR(K35A,E65D,K74Q,E80A,D82A,K130T,K135R)	140	35	8	58	40	SY46
SakSTAR(K74Q,K130T,K135R)	190	50	25	67	62	SY41
SakSTAR(T21A,K35A,E65Q,K74Q,K130A,K135R)	110	50	26	72	50	SY78
SakSTAR(E65Q,K74Q,K109A,K121A,K130A,K135R)	140	50	31	73	52	SY88
SakSTAR(E65Q,K74Q,D82A,S84A,K109A,K130A,K135R)	180	43	20	62	44	SY89

Table 8 - cont'd: SakSTAR variants with intact specific activity (≥ 100 kHU/mg) and ≤ 50 percent absorption of human antibodies elicited by treatment with wild-type SakSTAR

Variant	Spec. Act. (kU/mg)	SakSTAR patient plasma				Code
		Pool 10	Subpool B	Subpool C	Pool 40	
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R,+137A)	120	45	30	74	60	SY93
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R,K136A,+137K)	1,400	37	16	70	54	SY94
SakSTAR(E65Q,K74Q,D82A,S84A,E108A,K109A,K130A,K135R)	110	46	26	63	41	SY95

Antibody absorption ≤ 60 percent of wild-type SakSTAR are represented in bold type; $\geq 100,000$ HU/mg represented in bold type.

Table 2: Fibrinolytic properties of selected SakSTAR variants in human plasma in vitro

Compound	Fibrinolytic potency (C50 in $\mu\text{g/mL}$)	Residual fibrinogen at C50 (% of baseline)	Fibrinogenolytic potency (C50 in $\mu\text{g/mL}$)
SakSTAR	0.18 ± 0.01	93 ± 3.5	24 ± 3.6
SakSTAR(K74Q,E80A,D82A,K130T,K135R)	0.15 ± 0.01	97 ± 3.0	14 ± 3.2
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	0.24 ± 0.04	94 ± 10	29 ± 3.1
SakSTAR(K35A,E65D,K74Q,E80A,D82A,K130T,K135R)	0.11 ± 0.01	92 ± 3.0	20 ± 2.0
SakSTAR(E65Q,K74Q,N95A,E118A,K130A,K135R,K136A,+137K)	0.13	91	

The data represent mean \pm SD of 3 experiments.

C₅₀: amount of wild type or variant SakSTAR required for 50% clot lysis or 50% fibrinogen breakdown in 2 hrs.

Table 10: Pharmacokinetic parameters of the disposition of staphylokinase-related antigen from plasma following bolus injection of SakSTAR variants (100 µg/kg) in hamsters.

Variant	C ₀ (µg/mL)	A (µg/mL)	B (µg/mL)	t _{1/2} (α) (min)	t _{1/2} (β) (min)	V _C (mL)	AUC (µg.min.mL ⁻¹)	Cl _p (mL.min ⁻¹)
SakSTAR	0.8 ± 0.1	0.6 ± 0.1	0.2 ± 0.0	2.8	7.0	13 ± 1.0	4.6 ± 0.4	2.2 ± 0.2
SakSTAR(K74Q,E80A,D82A,K130T,K135R)	0.5 ± 0.1	0.4 ± 0.1	0.1 ± 0.0	2.0	10	20 ± 2.2	2.5 ± 0.3	4.1 ± 0.5
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	0.6 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	2.0	10	16 ± 1.1	2.8 ± 0.2	3.7 ± 0.3
SakSTAR(K35A,E65DK74Q,E80A,D82A,K130T,K135R)	1.1 ± 0.1	1.0 ± 0.1	0.1 ± 0.0	2.0	24	9.6 ± 0.7	6.4 ± 0.5	1.6 ± 0.1

Data are mean ± SEM of 4 experiments.

Table 11: Baseline characteristics and treatment outcome of the patients with peripheral arterial occlusion treated with SakSTAR, SakSTAR(K74Q,E80A,D82A,K130T,K135R) or SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)

Compound Patient Id.	Gender	Age (yrs)	Clinical ischemia	Locus of occlusion	Age of occlusion (days)	Length of occlusion (cm)	Recanalization by thrombolysis	Total dose of thrombolytic agent (mg)	Total duration of infusion (hrs)	Additional therapy
SakSTAR										
PUT	M	66	Subacute	Femoro-femoral graft	6	5	Complete	2	23	Stenting left IF artery
VERM	M	73	Acute	Right PA	2	6	Partial	13	23	Right upper leg amputation
GEIV	V	63	Restrain	Left SFA	10	5	Complete	8	7	PTA
POL	M	46	Subacute	Right SFA	30	50	Complete	22	29	Lumbar sympathectomie
BUE	F	53	Claudication	Right AF graft	1	15	Complete	10	13	Desobstruction
VII	F	75	Subacute	Left FT graft	2	34	Complete	7	10	PTA
REN	M	48	Restrain	Right IF graft	4	20	Complete	6.5	5	Left AF graft
COR	V	78	Acute	Left AFS	14	9	Complete	4	3	PTA
MAN	M	67	Restrain	Left tibial artery	1	6	Partial	6	5	-
STRA	M	66	Claudication	Right FP graft	14	16	Complete	19	26	-
VANH	M	38	Acute	Left radial artery	4	1	Complete	6	5	-
VANW	F	57	Acute	Right FP graft	1	25	Complete	20	24	New right FP graft
BRA	M	57	Acute	Left FT graft	1	30	Complete	25	43	-
DON	M	60	Claudication	Left FT graft	1	20	Complete	13	19	PTA + stenting
CAM	M	77	Restrain	Right SFA graft	8	30	Complete	27	44	FF graft
Mean ± SEM		62 ± 3.1			6.6 ± 2.1	18 ± 3.5		13 ± 2.1	19 ± 3.5	
SakSTAR(K74Q,E80A,D82A,K130T,K135R)										
IMB	M	66	Claudication	Left SFA	30	5	Complete	24	24	PTA
AZY	M	44	Subacute	Right C.I.A.	7	8	Complete	18	23	Stenting
VIN	M	51	Acute	Right E.I.A.	5	70	Complete	24	30	-
STRO	M	53	Claudication	Left FP junction	14	5	Partial	3.5	2	Aspiration thrombectomy, PTA
VERG	M	62	Restrain	Left SFA	20	6	Complete	19	25	FP bypass
GIE	M	76	Acute	Right FP bypass	2	15	Complete	8.5	17	-
Mean ± SEM		59 ± 4.7			13 ± 4.3	18 ± 10		16 ± 3.4	20 ± 4.0	
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)										
URB	M	57	Subacute	Right E.I.A.	4	8	Complete	8	6	Pseudo aneurysm, right AF graft revision
COM	M	59	Restrain	Right AF graft	7	65	Complete	16	22	-
HAC	M	70	Restrain	Left anterior tibial artery	7	15	Complete	12	14	-
DEW	F	76	Restrain	SFA	21	6	Complete	6	4	-
VAI	F	65	Subacute	Left PA	25	10	Partial	8	6	Aspiration thrombectomy
FIL	M	76	Claudication	Right SFA	28	8	Complete	24	31	PTA
Mean ± SEM		67 ± 3.4			15 ± 4.3	19 ± 9.4		12 ± 2.8	14 ± 4.4	

AF: aortofemoral; CABG: coronary artery bypass graft; CAD, coronary artery disease; CIA: common iliac artery; COPD: chronic obstructive pulmonary disease; DM: diabetes mellitus; EIA: external iliac artery; FF: femorofibular; FP: femoropopliteal; FT: femorotibial; IA: iliac artery; IF: iliofemoral; ocd: occlusion; PA: popliteal artery; PTA: percutaneous transluminal angioplasty; SFA: superficial femoral artery; TA: tibial artery; TF: tibiofibular; SC: subclavian.

Table 12: Absorption with SakSTAR variants of antibodies elicited with SakSTAR variants in patients with peripheral arterial occlusion

Treatment	Absorbant	Insolubilized compound		
		SakSTAR	SakSTAR(K74Q,E80A,D82A,K130T,K135R)	SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)
SakSTAR (Pool 40)				
	SakSTAR	95		
	SakSTAR(K74Q,E80A,D82A,K130T,K135R)	48		
	SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	57		
SakSTAR(K74Q,E80A,D82A,K130T,K135R) (Imb., Vin., Ver., Gie.)				
	SakSTAR	94	95	95
	SakSTAR(K74Q,E80A,D82A,K130T,K135R)	91	93	89
	SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	92	94	94
SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R) (Urb.)				
	SakSTAR	90	88	85
	SakSTAR(K74Q,E80A,D82A,K130T,K135R)	94	95	94
	SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R)	94	95	94

Data represent median values of the percent absorption with 250 nM absorbant, measured by residual binding to insolubilized compound.

* p= versus SakSTAR; ** p= versus SakSTAR(K74Q,E80A,D82A,K130T,K135R)); and p= versus SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R) by paired non-parametric test.

Table 13: Cysteine substitution variants of SakSTAR

Variant	Spec. Act. (kU/mg)	Dimerization level (%)	PEG derivatization	Clot lysis <i>in vitro</i> (C ₅₀ in µg/ml)	t1/2(α) (min)	Clp (ml/min)
SakSTAR	130	0	none	0.33	2.0	2.2
SakSTAR (K102C)	143	0	none	0.29	nd	nd
SakSTAR (K102C-PEG)	108	0	1	0.60
SakSTAR (K109C) monomeric	100	0	none	0.52	nd	nd
SakSTAR (K109C) dimeric	1,650	>60	none	0.17	3.6	0.52
	2,235	>95	none	0.12	nd	nd

APPENDIX

Table 14: Cysteine substitution variants of SakSTAR

Variant	Spec. Act. (kU/mg)	SakSTAR patient plasma				Code
		Pool 10	Subpool B	Subpool C	Pool 40	
SakSTAR(K96C)	790	95	95	95		
SakSTAR(K102C)	280	95	95	95		
SakSTAR(K109C)	1,900	95	95	94	90	
SakSTAR(K35C)						
SakSTAR(K74C)						
SakSTAR(.....)						
SakSTAR(K35A,E65D,K74Q,E80A,D82A,K109C,K130T,K135R)	600	46	32	56	36	SY100
SakSTAR(E65Q,K74Q,D82A,S84A,K109C,K130T,K135R)	1,100	54	53	77	41	SY102

Antibody absorption ≤ 60 percent of wild-type SakSTAR are represented in bold type; $\geq 100,000$ HU/mg represented in bold type.